THE REPLACEMENT OF A NATIVE COCCINELLID *COCINELLA NOVEMNOTATA*, BY AN INTRODUCED SPECIES, *COCCINELLA SEPTEMPUNCTAT:* ASSESSING IMPLICATIONS FOR PEST SUPPRESSION

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Evan Weston Hoki

August 2014

Abstract – Chapter 1

While the importation and release of foreign natural enemies in classical biological control programs can be cost-effective, the released control agent can have unintended effects, such as the displacement of native natural enemies. In this study we examined the effect of the replacement of a native coccinellid, the nine-spotted lady beetle (*Coccinella novemnotata*) by an exotic biological control agent, the seven-spotted lady beetle (*C. septempunctata*), on the ability of the coccinellid complex to control pest aphids. Specifically, we compared the consumptive and non-consumptive effect of each coccinellid species on aphids. The consumptive effect was measured by determining the functional responses for each lady beetle and calculating attack rate, handling time, and maximum feeding rate on a shared prey item, the pea aphid (*Acyrthosiphon pisum*) Non-consumptive effects were examined by assessing pea aphid dropping rates in response to foraging by the coccinellids on fava bean plants. Our results indicate that *C. septempunctata* has both a significantly greater consumptive effect and non-consumptive effect on *A. pisum* than *C. novemnotata.* Modeling indicates that *C. septempunctata* should have a greater impact on *A. pisum* populations but that its greater predatory efficiency could also lead to decreased stability in the interactions between predator and prey populations.

Abstract – Chapter 2

Numerous biological control programs within the United States have used coccinellids to control soft-bodied insect pests. While many of these programs have been successful, the importation of foreign natural enemies can have unintended effects on native species. In this study we focus on the exotic natural enemy, the seven-spotted ladybeetle (*Coccinella septempunctata*) and the displaced native, the nine-spotted ladybeetle (*C. novemnotata*). Specifically, we examined how the presence of a conspecific or heterospecific adult beetle and the density of aphid prey affected foraging behavior. In addition we determined if olfactory cues from aphids or fava bean plants (*Vicia faba*) played a role in foraging decisions for these two species. Our results indicate that *C. novemnotata* was strongly affected by *C. septempunctata* presence when foraging and less affected by conspecifics, whereas *C. septempunctata* avoided both heterospecifics and conspecifics equally. The effect of aphid density affected both species differently with *C. septempunctata* having a lower retention time on an infested pea plants than *C. novemnotata* at the lowest aphid density. Using a Y-tube olfactometer, we found no effect of aphid or plant olfactory cues being utilized by either *C. novemnotata* or *C. septempunctata.*

BIOGRAPHICAL SKETCH

Evan Hoki earned his Bachelor of Science in Entomology and in Plant Science from Cornell University in 2010. In 2012 he returned to Cornell University to work towards a Master of Science degree in Entomology.

Evan was a recipient of the SUNY Diversity Fellowship, which helped him work towards his degree. After graduation Evan will be serving as the Manager of Live Collections and Chief Entomologist at The Strong museum in Rochester, New York.

ACKNOWLEDGMENTS

I would like to thank my coauthors on these two chapters, John Losey and Todd Ugine, for working tirelessly to help me design, write, and present this research. I would also like to thank Alison Power for her help with the experimental design. Their guidance was invaluable in helping me grow as a scientist. I would also like to thank the State University of New York system for awarding me the SUNY Diversity Fellowship.

TABLE OF CONTENTS

CHAPTER 1

Comparing the consumptive and non-consumptive effects of a native and introduced lady beetle on pea aphids (*Acyrthosiphon pisum*)

1.0 Introduction

1.1 Background

Classical biological control, where an exotic natural enemy is imported to control a pest species, can be an effective, low-cost method for the sustained management of pest populations (Debach and Rosen, 1991; Barratt et al., 2000). Since 1888, over 700 insect biocontrol agents have been introduced into the United States (Greathead and Greathead, 1992). In California alone, major biological control projects saved over $500 million between 1900-1985 (Debach and Rosen, 1991). Additionally, classical biological control projects have a high benefit:cost ratio with some projects exceeding 100:1. In Australia the average estimated benefit:cost ratio of biological control projects was 10.6:1, which exceeded the ratio of chemical control projects (2.5:1) (Driesche and Bellows, 1996). Some classical biological control programs have been spectacularly successful, one example being the control of the cottony-cushion scale, *Icerya purchasi* Maskell by the vedalia lady beetle, *Rodolia cardinalis* Mulsant. It is estimated that this project cost less than $5,000 to implement and has saved the citrus industry millions of dollars annually (Debach and Rosen, 1991).

While there are numerous examples of the successful implementation of classical biological control, there are also examples of biological control agents having an adverse impact on native flora and fauna. Stiling and Simberloff (2000) estimate that 16% of all classical biological control attempts within the United States have had a negative impact on non-target organisms.

Coccinellids are commonly used as classical biological control agents to control soft-bodied pests like aphids in agricultural systems. The seven-spotted lady beetle, *Coccinella septempunctata L.*, is one of the many coccinellids that were imported into the United States for the biological control of aphids from 1957 to 1990 (Schaefer et al., 1987, Obrycki et al., 2000) and is believed to have become established in or around 1973. By 1987 the range of *C. septempunctata* had expanded to include much of the United States (Schaefer et al., 1987). *C. septempunctata* tends to be more voracious and larger than many of its native counterparts and because of this, it was expected to be an excellent biological control agent of aphids (e.g. Raak-van den Berg et al., 2012; Elliott et al., 1996; Gardiner et al., 2011). However, since the introduction of *C. septempunctata*, there has been a shift in the relative abundance of many native coccinellids. For instance, there has been a decline in some species such as the nine-spotted lady beetle, *Coccinella novemnotata* Herbst, the two-spotted lady beetle, *Adalia bipunctata* L. , and the transverse lady beetle, *Coccinella transversoguttata* F., (Elliott et al., 1995; Obrycki et al., 2000), while other species, such as the spotted lady beetle, *Coleomegilla maculata* De Geer, have become more common.

C. novemnotata is a species of particular interest because it is a congeneric species of *C. septempunctata* and they are thought to occupy the same ecological niche (Losey et al., 2012, Harmon et al., 2007). Prior to the establishment of *C. septempunctata, C. novemnotata* was one of the most commonly collected coccinellids in the United States (Gordon, 1985) and was widespread across the United States (Wheeler & Hoebeke, 1995; Harmon et al., 2007). It has been proposed that its disappearance could be due to displacement by *C. septempunctata* (Wheeler & Hoebeke, 1995; Losey et al., 2012), although it is also possible that *C. novemnotata* declined for other reasons and then *C. septempunctata* expanded to fill the empty niche.

Regardless of whether *C. septempunctata* is a "driver" of community change or a "passenger" (Didham et al., 2005), its replacement of *C. novemnotata* as one of the prominent natural enemies of aphids in the United States could change how the coccinellid complex interacts with aphids, which would impact biological control in agricultural systems.

The effect of a biological control agent on its prey can be assessed in terms of consumptive and non-consumptive effects. Consumptive effects occur when a predator feeds directly upon a prey item. Non-consumptive effects are those that reduce prey fitness, unrelated to death by consumption. Some of these effects include prey defensive and behavioral responses (Fill et al., 2012). Often, the non-consumptive effects that natural enemies inflict on prey populations can cause an equal or greater loss of fitness than the consumptive effects (Fill et al., 2012; Preisser et al., 2005). In the coccinellid-aphid system, the consumptive effect on aphids would be the direct consumption of the aphids. A non-consumptive effect would be the modification of aphid behavior in any way that affects its fitness. For instance, many studies have demonstrated that coccinellids and other aphidophagous predators can elicit a dropping response in the pea aphid, *Acyrthosyphon pisum* Harris, where the aphids fall from the plant to avoid predation (Evans 1976; Roitberg and Myers, 1978; Roitberg et al., 1979; Losey and Denno, 1998). Dropping is the most common escape mechanism employed by *A. pisum* (e.g. Montgomery & Nault, 1977). However, their propensity to drop is affected by a variety of factors such as temperature, humidity, aphid density, and the size the predator (Dixon 1958; Brodsky & Barlow, 1986; Losey and Denno, 1998; Evans 1976). While dropping behavior is an effective method for avoiding aphidophagous predators, under certain conditions, such as very warm, dry weather, dropping behavior can be costly and result in high aphid mortality (Roitberg and Myers, 1977; Ruth et al., 1975).

In this study we compared the consumptive and non-consumptive effects of *C. novemnotata* and *C. septempunctata* in order to gain insight into how the replacement of a native coccinellid by an exotic species will impact aphid populations. The consumptive effect was determined for both lady beetle species by determining their functional response, which is the relationship between resource density and consumption rate. It has been proposed that functional responses can be used as a means of comparing the relative impacts of invasive and native species on a prey population (Bollache et al., 2008; Dick et al., 2013). Other sources caution that several factors in the field add complexity and can cause predictions based on laboratory-based functional response studies to be inaccurate (Lester and Harmsen 2002). Predation rates in the field are difficult to measure so there are few comparisons of laboratory and field-based rates, but one surrogate for predation rate in the field is predator distribution, as optimal foraging theory would predict that predators should concentrate their efforts on prey that offer the highest nutritional value (Roger et al. 2001). Finlayson et al. (2010) found that their laboratory predation rates accurately predicted presence and density associated with various aphid species in the field. This provides evidence that, at least for coccinellids, predation rates measured in the laboratory do reflect patterns in the field.

The non-consumptive impact of each species was determined by observing *A. pisum* dropping rates in response to the presence of adult coccinellids of both species. Due to the potential negative consequences of dropping behavior on aphid fitness (Roitberg and Myers, 1977; Ruth et al., 1975), the non-consumptive effects of each coccinellid species has the potential to further differentiate their relative impacts on aphid populations.

1.2 Objectives

Although the "replacement" of *C. novemnotata* by *C. septempunctata* has been evident for decades, *C. novemnotata* had become so rare that it was not possible to conduct a full comparison of its impact on *A. pisum* with the impact of *C. septempunctata*. Recently, through the efforts of volunteers in the Lost Ladybug Project (www.lostladybug.org), stable populations of *C. novemnotata* have been identified and laboratory colonies have been established making a full comparison possible. We initiated this study to help provide insight into how replacement of *C. novemnotata* by an exotic congeneric species, *C. septempunctata*, would affect aphid populations in agricultural systems.

2.0 Methods

2.1.0 Rearing Methods and Origin of Lady Beetles

Adult *Coccinella septempunctata* and *C. novemnotata* were collected from Oregon in the summer of 2012 to start laboratory colonies. An additional *C. novemnotata* colony was started in 2012 from adults collected on Long Island, New York. Adult beetles and larvae were maintained separately in 44 mL plastic cups that contained a single piece of paper towel (6x2 cm) to serve as cover. Beetles were provided an *ad libitum* diet of mixed-aged *A. pisum* that had been produced on fava bean (*Vicia faba* L.) plants. Old and dead aphids were removed from cups daily and the paper towel replaced every 2-3 days. The rearing room was kept at 23 ± 2°C with a 16:8 (L:D) light cycle. A more detailed description of the rearing process can be found in Losey et al. (2012).

2.2.0 Consumptive effect test

To compare the consumptive effect of *C. septempunctata* and *C. novemnotata* populations on aphids, we conducted a study to determine their functional response. Individual adult virgin beetles (mean ± SE day old adults: 12.6 ± 0.40 days) in 44 ml plastic cups were provided a range of aphid densities, the range of which differed by species. Both male and female lady beetles were tested. *C. septempunctata* were provided 10, 15, 20, 25, 30, 35, or 40 third or fourth instar aphids and *C. novemnotata* were provided 10, 15, 20, 25, and 30 aphids. Preliminary tests indicated that adult *C. novemnotata* and *C. septempunctata* did not consume more than 30 and 40 third-instar aphids per day, respectively. These densities were chosen as the upper limit for the number of aphids provided beetles per day in our trial. The aphid density is expressed as aphid level per 44mL.

Prior to the start of each trial adult lady beetles were sexed, weighed and then starved for 24h. Beetles were maintained singly in 44 ml cups in an incubator at 23.3°C ± 0.05 and 42.2% ± 0.28 relative humidity. The following day aphids were added to each cup along with a 2x5cm paper towel and 100uL of tap water. Five to six holes were poked into the cap with a metal probe to minimize condensation. This amount of water and ventilation greatly improved the survivorship of aphids as compared to our preliminary trials. After 24h, the number of surviving, attacked, and dead aphids were counted. Any aphid that was still alive but had been attacked (as determined by melanization or noticeable bites) was put into one of two categories: 1) attacked – any aphid still alive with its head and at least 70% of its former body mass intact; insects within this category were alive and active, 2) moribund – any aphid still moving, but missing its head, or more than 30% of its body; these insects were usually lethargic or inert, discolored, and showing clear signs of poor health. Aphids within the first category could potentially reproduce

or harm the plant, while aphids within the second category were assumed to be dying or to have lost their reproductive capacity. Aphids that appeared dead but were neither eaten nor attacked were counted and were assumed to have died from natural causes. These aphids tended to be immobile and discolored. The number of aphids consumed in each trial was determined by subtracting the number of surviving aphids from the total amount offered in that trial. Aphids in the attacked category were counted as surviving aphids while aphids in the moribund category were counted as consumed. An additional set of two control cups without coccinellids were set up identically to those containing beetles to measure background mortality at each aphid density tested. Between 14 and 29 replicate cups of each aphid density and coccinellid treatment were monitored over a 3-month period.

We found no significant differences between the weights or aphid consumption of the two *C. novemnotata* populations therefore we pooled the data ($P = 0.582$).

2.1.1 Analysis of the consumptive effect

The functional response of each lady beetle species was generated using R Version 2.15.2012-10-21 (R Core Team, 2012) with aphid density as our dependent variable and the number of aphids killed as our response variable. To test whether each coccinellid species was displaying a Type II or Type III functional response, we used a logistic regression to test for a negative linear coefficient. Due to the 100% consumption rates displayed by *C. septempunctata* at lower aphid densities, the initial functional response approximated a Type III curve. To correct for this, we excluded the 10 and 15 aphid levels from *C. septempunctata,* which changed it into a Type II functional response. This was necessary in order to compare the two species using the Roger's equation for prey depletion (Bolker 2008).

Our experiment yielded a single value for the attack rate (*a*), handling time (*h*), and maximum estimated feeding rate (1/*h*T) for each coccinellid species, which were calculated using the Roger's equation for prey depletion as described in Bolker (2008). The general approach to our analysis, with minor modifications, was similar to that outlined in Dick et al. (2013). Bootstrapping was used to generate additional estimates (n=50) for attack rate, handling time, and maximum feeding rate, so that they could be compared using a Student's *t* test, which was performed with JMP Pro version 10 (SAS Institute, 2013)(Dick et al. 2013).

Using the bootstrapped values for attack rate and handling time, the efficacy of each coccinellid as a biological control agent was quantified by estimating the *q*-value (the ratio of the equilibrium prey population with and without a predator population present (Beddington et al. (1978)) using a simulation model constructed by Sharov (Last accessed: November 2014). Additional values needed to calculate the *q*-value were: the carrying capacity of the prey population (20, estimated from the number of aphids that would fit on the length of stem in a 44mL cup), the carrying capacity of the predator population per aphid per day (0.03 for both *C. septempunctata* and *C. novemnotata*, derived using unpublished data from Kopco) and the growth rates for both the *A. pisum* and the coccinellids (0.38 and and 0.2 respectively, using data from Legrand and Barbosa (2000) and ElHag and Zaitoon (1996)). Coccinellid growth rates were calculated using the formula: $r_m = (\ln(R_0))/T$, with R_0 being the replacement rate per generation, and T being the mean period require for progeny production (Wyatt and White, 1977; Rodriguez-Saona and Miller, 1995; Legrand and Barbosa, 2000). The values for R_0 and T were taken from ElHag and Zaitoon (1996) for *C. septempunctata* and *C. novemnotata*. Additional *q*-values were generated via bootstrapping (n=50), and were then compared using a Student's *t* test.

To ensure coccinellid sex ratios were not unbalanced, we performed a chi-square test using R Version 2.15.2012-10-21 (R Core Team, 2012). This was necessary due to the differential aphid consumption rate of each sex.

We found no significant differences between the weights or aphid consumption of the two *C. novemnotata* populations ($P = 0.582$) and therefore pooled the data.

2.3.0 Non-consumptive effect

Aphid dropping behavior in response to the presence of an adult coccinellid was monitored to estimate the impact of *C. novemnotata* and *C. septempunctata* on this non-consumptive effect. One-week old *V. faba* seedlings were transplanted into inverted 237mL funnels filled with Lambert soil (Quebec City, Canada) LM-series professional growing media. The base of this structure was sealed with a 100x15mm petri dish that was held in place with Parafilm (Pechiney Plastic Packaging Company, Chicago, Illinois).

A diagram of the apparatus used for testing the non-consumptive effects of lady beetle on aphids can be viewed in Figure 1. A ring stand was mounted with two rings. After 4-5 days of additional growth the funnels containing plants were placed onto the top ring of the ring stand. The bottom ring was outfitted with a 1.89L funnel that was coated with Insect-a-Slip (BioQuip, Rancho Dominguez, CA) to prevent aphids that dropped from the plant from climbing out. Additionally, a 4cm diameter plastic mesh circle (hole size 3x3mm) was placed at the funnel exit that allowed aphids to pass through the holes but prevented coccinellids from falling through. Below the 1.89L funnel was a 125mL Erlenmeyre flask with 25mL of soapy water that caught the fallen aphids. The entire apparatus was housed within a fine wire mesh cage.

Prior to the experiment, the opening from which the stem of the plant emerged from the inverted funnel was sealed with parafilm to prevent aphids from traveling down into the funnel.. Then an aphid platform (Figure 1b) was constructed by applying Insect-a-Slip to the inside walls of 44.4mL cups. Using a heated cork borer, a 16mm (diameter) hole was burned into the center of each cup, which was then cut once radially and placed around the stem of the plant. Cotton was wrapped around the base of the stem to further plug the hole and prevent aphids from escaping out of the bottom of the cup. The radial cut in the cups, were then taped shut to keep it in place.

Twenty-four hours prior to the introduction of lady beetles into cages, 50 third or fourth-instar aphids were placed into each platform and were allowed to climb onto the plant. The following day, the number of aphids that had died within each platform was recorded, the platforms were removed and the number of aphids that had fallen off of the plant and into the soapy water was recorded. By recording these numbers it was possible to calculate the number of aphids that remained on the plant prior to the introduction of lady beetles. One adult lady beetle (either *C. novemnotata* or *C. septempunctata*), that had been sexed, starved for 24h to ensure maximum voracity and subsequently weighed, was added to the base of the plant using a small paintbrush. The beetles were removed 3h post-introduction into the cages and the number of newly fallen aphids was recorded. The experiment was conducted in a greenhouse that was maintained at 22.7°C ± 0.73 with 41.7% ± 4.68 relative humidity.

Figure 1a-b: The experimental set up for the dropping experiment. 1b) The platform used to place *A. pisum* on the plant

2.3.1 Analysis of the non-consumptive effect

The effect of coccinellid species on *A. pisum* dropping behavior was analyzed with logistic regression using the statistical package JMP Pro 10 (SAS Institute, 2013). The number of aphids "dropped" and "not dropped" were counted for each replicate plant. To simulate the effect of dropping on a population basis (as opposed to an individual plant basis) the total number of aphids that "dropped" and "not dropped", across all replicates, was used for the logistic regression.

3.0 Results

3.1 Consumptive effect test

C. septempunctata consumed significantly more aphids at each density than *C. novemnotata* (Figure 2). Consumption rates were significantly affected by both aphid density (*x2*

= 371.25, $P < 0.0001$) and species ($x2 = 247.96$, $P < 0.0001$), and there was no interaction between aphid density and lady beetle species ($x2 = 0.73$, $P = 0.73$).

C. septempunctata exhibited a significantly higher attack rate than *C. novemnotata* ($t = 9.41$, $P < 0.0001$), and a lower handling time ($t = -19.58$, $P < 0.0001$). Additionally, *C. septempunctata* had a higher estimated maximum feeding rate than *C. novemnotata* ($t = 15.82$, $P < 0.001$). *C. septempunctata* displayed a significantly lower *q*-value than *C. novemnotata* (Table 1) ($t = -14.19$, $P < 0.0001$).

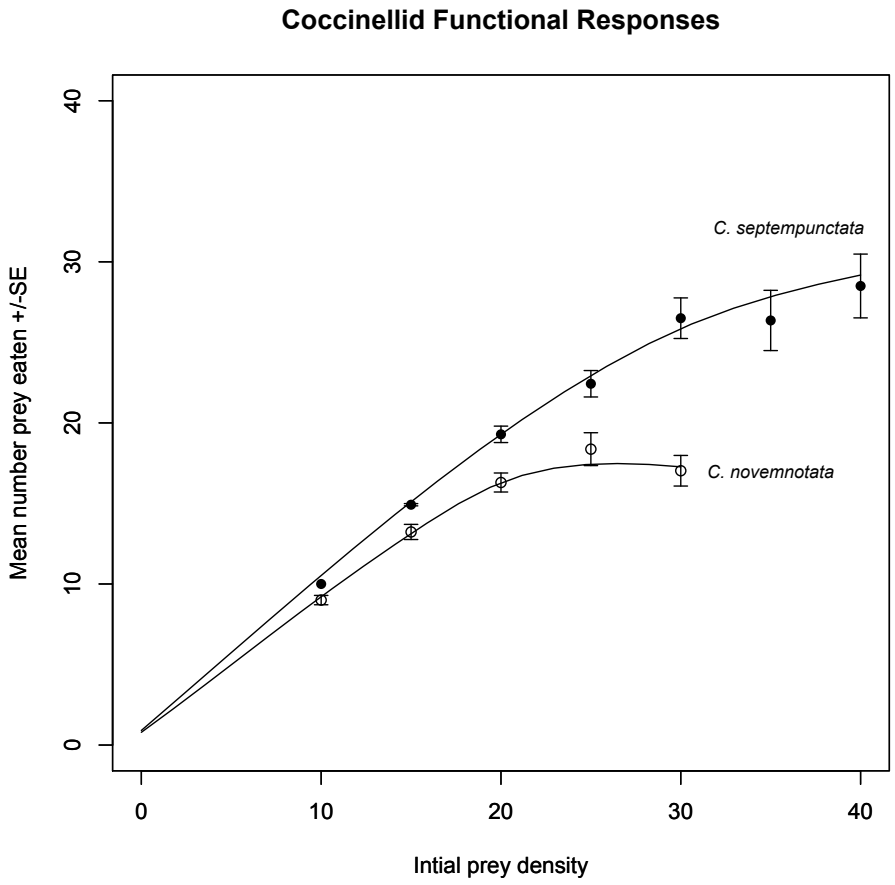

Figure 2: The functional responses of *C. septempunctata* and *C. novemnotata* on *A. pisum*, with the mean values and standard error.

3.2 Non-consumptive effect test

C. septempunctata induced higher *A. pisum* dropping rates than *C. novemnotata* (Figure 3). Aphid dropping rates were affected by both sex ($x2 = 24.93$, $P < 0.0001$) and species ($x2 = 18.01$, $P < 0.0001$), however, the interaction between the two was not significant ($x2 = 3.66$, $P = 0.056$).

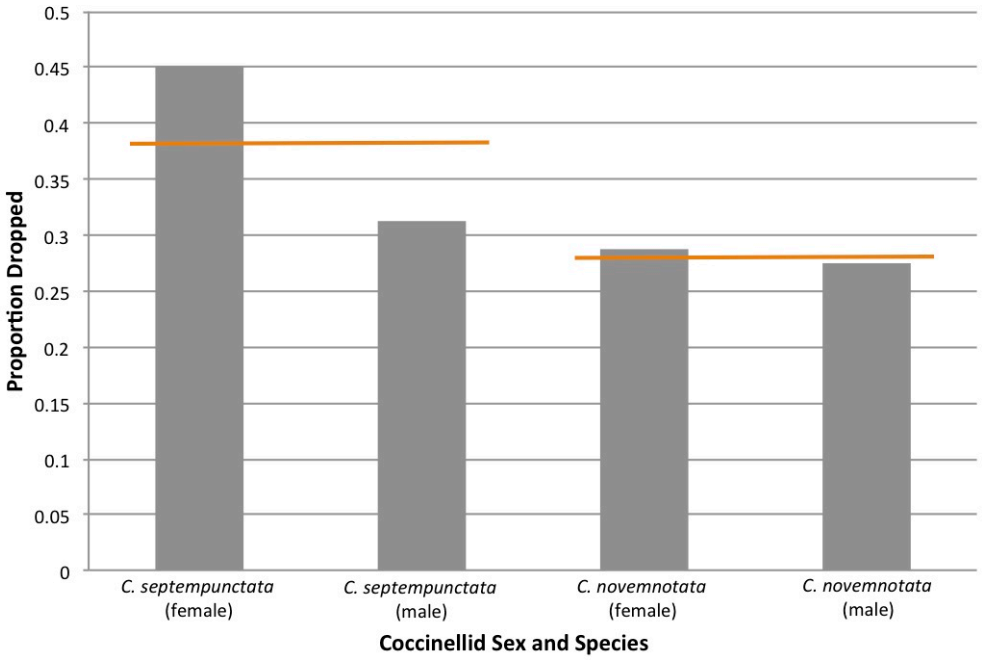

Figure 3: The Proportion of *A. pisum* dropped relative to coccinellid sex (Female or Male) and species (C7= *C. septempunctata;* C9 = *C. novemnotata*). The line between the species treatments is the average of the proportion dropped between the two sexes (0.385 for C7, 0.275 for C9).

4.0 Discussion

Our results show that *C. septempunctata* had both a higher predatory efficiency and it elicited a stronger dropping response in *A. pisum* populations as compared to *C. novemnotata*. These results provide evidence that the replacement of *C. novemnotata* by *C. septempunctata* within the coccinellid complex could result in increased consumptive and non-consumptive pressure on *A. pisum* populations.

Changes like this are potentially important because coccinellid communities usually consist of a few dominant species. Often, just two to four species represent 90% of the individuals within a system (Hodek and Honek, 1996). Until recently, *C. novemnotata* was one of the most common coccinellids north of Mexico (Gordon, 1985) and a key player in the coccinellid complex. It is unclear whether or not the congeneric *C. septempunctata* is directly responsible for the decline in *C. novemnotata* (Harmon et al., 2007), but since its establishment between 1970-1980, *C. septempunctata* has rapidly spread across the United States (Wheeler and Hoebeke, 1995; Obryki et al., 2000). Fothergill and Tindall (2010) state that *C. novemnotata* populations were declining in Missouri years before *C. septempunctata* had arrived, and conclude that it is unlikely that *C. septempunctata* is responsible for the decline. However, it is possible that *C. septempunctata* was present in Missouri and was impacting native species years before it was detected (Losey et al., 2012). *C. septempunctata* which has been described as voracious, polyphagous, and aggressive (Wheeler & Hoebeke, 1995; Gordon, 1985) has been observed preying on other predators from the same guild such as lacewings (Sengonca & Frings, 1985) and other coccinellid larvae (Raak-van den Berg et al., 2012) in laboratory studies. In interactions with various other native coccinellids, such as *A. bipunctata* (Raak-van den Berg et al., 2012) and *C. novemnotata* (Turnipseed et al., unpublished data) *C. septempunctata* tended to dominate. While many native coccinellids have decreased in abundance since the introduction of *C. septempunctata*, other species such as *C. maculata* have become more common (Elliott et al., 1996; Obrycki et al., 2000). Even if *C. septempunctata* is not causing the decline of *C. novemnotata*, there is evidence that its presence has changed the makeup of the coccinellid complex in the United States (e.g. Elliot et al., 1996; Evans, 1991; Harmon et al., 2006; Obrycki et al., 2000). Based on our results, this change in the relative abundance of different coccinellid

species within the ecosystem could cause a corresponding change in the ability of the coccinellid complex to control pest populations

It is difficult to predict how the changing densities of coccinellid species will affect agricultural and natural systems. One method that is used to predict the negative impact an invasive species might have on native communities is the comparison of functional responses between the exotic species and a functionally similar native species. Specifically, elements of predatory efficiency such as attack rate (a), handling time (h), and maximum feeding rate ($1/hT$) can be used as a gauge of relative impact on prey populations with higher values of a and $1/hT$ and lower values of h indicating a greater ecological impact (Bollache et al., 2008; Dick et al., 2013). The functional responses generated for this manuscript suggest that the proliferation of *C. septempunctata* could have a large impact on aphid, and consequently, native coccinellid communities. Breaking down the functional response into its component elements measured in this experiment give some additional insight into how *C. septempunctata* spread so rapidly and replaced the once common *C. novemnotata.*

While functional responses have been proposed as a method of predicting the impact exotic species could have on native communities, there are factors that can affect the accuracy of this method. Kestrup et al. (2011) demonstrated that in amphipod systems, intraguild predation and the environment are important factors to consider. In their experiment, they found that, while the one amphipod species had lower predatory efficiency under certain aquatic conditions, it became dominant in intraguild predation events. These events allowed the amphipod to persist even though it consistently had a lower functional response then other amphipod species. In the coccinellid-aphid complex, not only does *C. septempunctata* have a higher functional response than *C. novemnotata*, it also dominates in intraguild predation events. (Turnipseed, unpublished

data). Under such conditions, it would be understandable if *C. novemnotata* populations were displaced by *C. septempunctata,* especially due to the similarity of their ecological niches (Harmon et al., 2007)

It is unclear whether or not the displacement of *C. novemnotata* by *C. septempunctata* would lead to greater suppression of aphid pests. The *q*-value can be used to quantify a predator's efficacy as a biological control agent (Beddington et al., 1978). A *q*-value that is less than 0.5 is often indicative of successful biological control (Beddington et al., 1978; Sharov n.d.). Our data suggest that, both *C. novemnotata* and *C. septempunctata* are effective biological control agents. However, the lower *q*-value of *C. septempunctata* indicates that it may be a more efficient control agent then *C. novemnotata*. Interestingly, *C. septempunctata* was so effective it completely eliminated the aphid population 20% of time during our bootstrapped simulations, while *C. novemnotata* never drove the prey to local extinction. This could be due to the small space of the arena, which was used to calculate the model and the fact that no organisms can enter or leave the space. However, if this pattern holds true outside laboratory studies, it would indicate that, while *C. septempunctata* is very effective for biological control, its relationship with *A. pisum* is unstable. Conversely, *C. novemnotata* may be less effective as a control agent than *C. septempunctata,* but because it never completely eliminates *A. pisum*, it may have a more stable relationship with its prey population.

Additional evidence for the higher efficacy of *C. septempunctata* as a biological control agent was provided by our analysis of the dropping response of *A. pisum,* which was found to be greater in the presence of *C. septempunctata* than with *C. novemnotata.* However, even with the clear difference in the pea aphid's dropping response to the two predators, determining the impact of higher dropping rates on pea aphid population dynamics can be complex. The

effectiveness of aphid dropping as an escape mechanism varies. In warm, dry climates dropping can result in high mortality (Ruth et al., 1975; Roitberg and Myers, 1977), reducing its efficacy of the escape mechanism. However, under other circumstances, where the predator:aphid size ratio is large, dropping can be more beneficial then remaining on the plant (Evans, 1976). When confronted by a predator, aphids can choose to run, kick, or drop from the plant (Dixon, 1958; Evans, 1976). A study by Evans (1976) demonstrated that, when *A. pisum* encounters a predator of equal or greater size (e.g. *Anthocoris nemorum* L.), running and pulling away became less successful in deterring predators. Because of this, dropping was the only option. Additionally, an experiment by Dixon (1958) showed this trend in coccinellid systems with the aphid, *Microlophium evansi* Theobald, where larger coccinellid larvae induced higher aphid dropping rates. This pattern held true for our system as well, with the larger *C. septempunctata* triggering a greater dropping response than *C. novemnotata.* Although the optimal defensive response to a given predator can vary, the greater danger facing an aphid that remains on a plant where *C. septempunctata* is foraging coupled with the ostensibly equal rates of mortality if they do drop, indicate that a higher proportion of aphids will be killed either through consumption on the plant or alternate mortality sources (e.g. desiccation, consumption) off the plant. Thus, we predict that the overall impact of *C. septempunctata* on pea aphid populations will be substantially greater than that of *C. novemnotata*, the species it has replaced.

Beyond suppressing aphid populations, dropping behavior induced by coccinellids and other predators may also play a role in aphid dispersal. Roitberg et al. (1979) demonstrated that, unless the plant is in decline, *A. pisum* rarely moved between plants. However, if there was a predator present, the aphids changed plants frequently. Because of this they hypothesized that predator presence may have an important role in mediating aphid dispersal. If this is the case, the

higher dropping rates found in our *C. septempuncatata* treatment could serve to facilitate the dispersal of aphids (Roitberg et al., 1979) and by extension, the transmission of aphid-vectored plant diseases (Evans, 1991).

In this study we went beyond comparison of the overall functional response in several important aspects to provide additional insight into how the introduction this exotic organism may impact agricultural systems. These include calculation of q-values for each coccinellid species and quantification of both consumptive and non-consumptive effects. The functional response served as an indicator of the potential impact of an exotic species. We found that *C. sepetempunctata* had higher predatory efficiency and a stronger consumptive impact on *A. pisum* populations than *C. novemnotata,* suggesting that it may be more effective at controlling aphid populations. This data was further supported by the lower *q*-value calculated for *C. septempunctata.* A decline in the density of pea aphids in Utah associated with the invasion of *C. septempunctata* supports this prediction (Evans, 2004)

However, we also found that *C. septempunctata* is so effective that it has the potential to cause aphid populations to crash, indicating that it may have a less stable relationship with *A. pisum* than *C. novemnotata* does. Although our separate experiments do not allow us to quantitatively combine predation and dropping rates, the greater impact on aphid populations and the associated greater instability estimated for *C. septempunctata* would theoretically be exacerbated by the higher rates of dropping caused by this exotic predator. Clearly, many other factors such as the ability to locate patches of prey, the ability to locate viable overwintering sites, and the ability to disperse over large distances to find these resources will play a large role, but based on our data we predict that a complex of coccinellids that contains *C. septempunctata*

instead of *C. novemnotata* will have a greater ability to suppress pea aphid populations but could potentially be less stable.

REFERENCES

Brodsky, L.M., and Barlow, C.A., 1986. Escape responses of the pea aphid, *Acyrthosiphon pisum* Harris)(Homoptera: Aphididae): influence of predator type and temperature. Can J Zool *64*, 937–939.

Beddington, J.R., Free, C.A. and Laston, J.H., 1978. Characteristics of successful natural enemies in models of biological control of insect pests. Nature *273,* 513-519.

Bollache, L., Dick, J.T.A., Farnsworth, K.D., Montgomery, W.I., 2008. Comparison of the functional responses of invasive and native amphipods. Biol Lett *4,* 166-169.

Bolker, B.M., 2008. Ecological models and data in R. (Princton University Press).

Debach, P., and Rosen, D., 1991. Biological control: definition and scope (Cambridge University Press).

Didham, R., Tylianakis, J., Hutchison, M., Ewers, R., and Gemmell, N., 2005. Are invasive species the drivers of ecological change? Trends Ecol Evolut *20*, 471–474.

Dick, J.T.A., Gallagher, K., Avlijas, S., Clarke, H., Lewis, S., Leung, S., Minchin, D., Caffrey, J., Alexander, M., Maguire, C., et al., 2013. Ecological impacts of an invasive predator explained and predicted by comparitive functional responses. Biol Invasions *15*, 837–846.

Dixon, A.F.G., 1958. The escape responses shown by certain aphids to the presence of the coccinellid Adalia decempunctata (L.). Trans R Entomol Soc Lond *110*, 319–334.

Dixon, A.F.G., 1959. An experimental study of the searching behaviour of the predatory coccinellid beetle Adalia decempunctata (L.). J Anim Ecol 259–281.

Driesche, R., and Bellows, T.J., 1996. Biol Control (Chapman & Hall).

Easteal, S., 1981. The history of introductions of *Bufo marinus* (Amphibia: Anura); a natural experiment in evolution. Biol J Linnean Soc *16*, 93–113.

ElHag, E.T., and Zaitoon, A., 1996. Biological parameters for four coccinellid species in central Saudi Arabia. Biol Control *7*, 316–319.

Elliot, N., Kieckhefer, R., and Kauffman, W., 1996. Effects of an invading coccinellid on native coccinellids in an agricultural landscape. Oecol *105*, 537–544.

Evans, E.W., 1991. Intra versus interspecific interactions of lady beetles (Coleoptera: Coccinellidae) attacking aphids. Oecol *87*, 401–408.

Evans, E. W. 2004. Habitat displacement of North American ladybirds by an introduced species. *Ecology*, *85*, 637-647.

Evans, H.F., 1976. The role of predator-prey size ratio in determining the efficiency of capture by *Anthocoris nemorum* and the escape reactions of its prey, Acyrthosiphon pisum. Ecol Entomol *1*, 85–90.

Fill, A., Long, E.Y., and Finke, D.L., 2012. Non-consumptive effects of a natural enemy on a non-prey herbivore population. Ecol Entomol *37*, 43–50.

Fothergill, K., Tindall, K.V., 2010. Lady beetle (Coleoptera: Coccinellinae) occurrences in Southeastern Missouri agricultural systems: differences between 1966 and present. Coleopts Bull *64,* 379-382.

Funk, J.L., and Vitousek, P.M., 2007. Resource-use efficiency and plant invasion in low-resource systems. Nature *446*, 1079–1081.

Gardiner, M.M., O'Neal, M.E., and Landis, D.A., 2011. Intraguild predation and native lady beetle Decline. PLOS ONE *6*, e23576.

Greathead, D.J., and Greathead, A.H., 1992. Biological control of insect pests by insect parasitoids and predators: the BIOCAT database. Biocon News Info *13*, 61N–68N.

Gordon, R.D., 1985. The Coccinellidae (Coleoptera) of America North of Mexico. J New York Entomol S *93,* 1-912.

Harmon, J.P., Stephens, E., and Losey, J., 2007. The decline of native coccinellids (Coleoptera: Coccinellidae) in the United States and Canada. J Insect Conserv 85–94.

Hodek, I., Honek, A., 1996. Ecology of Coccinellidae. (Kluwer Academic Publishers)

Kestrup, A.M., Dick, J., Ricciardi, A., 2011. Interactions between invasive and native crustaceans: differential functional responses of intraguild predators towards juvenile hetero-specifics. Biol Invasions *13,* 731-737.

JMP pro, Version 10. SAS Institute Inc., Cary, NC, 1989-2013.

Legrand, A., and Barbosa, P., 2000. Pea Aphid (Homoptera: Aphididae) Fecundity, Rate of Increase, and Within-Plant Distribution Unaffected by Plant Morphology. Environ Entomol *29*, 987–993.

Losey, J.E., and Denno, R.F., 1998. Positive predator-predator interactions: enhanced predation rates and synergistic suppression of aphid populations. Ecology *79*, 2143–2152.

Losey, J., Perlman, J., Kopco, J., Ramsey, S., Hesler, L., Evans, E., Allee, L., and Smyth, R., 2012. Potential causes and consequences of decreased body size in field populations of Coccinella novemnotata. Biol Control *61*, 98–103.

Losey, J.E., Perlman, J.E., and Hoebeke, E.R., 2007. Citizen scientist rediscovers rare nine-spotted lady beetle, Coccinella novemnotata, in eastern North America. J Insect Conserv *11*, 415–417.

Montgomery, M.E., and Nault, L.R., 1977. Comparative response of aphids to the alarm pheromone,(E)-ß-Farnesene. Entomol Exp Appl *22*, 236–242.

Obrycki, J.J., Elliot, N.C., and Giles, K.L., 2000. Coccinellid introductions: potential for and evaluation of nontarget effects. In Nontarget Effects of Biological Control, P.A. Follet, and J.J. Duan, eds. (Kluwer Academic Publishers).

Pimentel, D., Lach, L., Zuniga, R., and Morrison, D., 2000. Environmental and economic costs of nonindigenous species in the United States. BioScience *50*, 53–65.

Preisser, E.L., Bolnick, D.I., and Benard, M.F., 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. Ecology *86*, 501–509.

R Core Team, 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Rodriguez-Saona, C., and Miller, J., 1995. Life history traits of *Hippodamia convergens* (Coleoptera: Coccinellidae) after selection for fast development. Biol Control *5*, 389–396.

Rogers, D.,1972. Random Search and Insect Population Models. J Anim Ecol *41*, 369–383.

Roger, C., Coderre, D., Vigneault, C. and Boivin, G. (2001), Prey discrimination by a generalist coccinellid predator: effect of prey age or parasitism?. Ecol Entomol *26*, 163–172.

Roitberg, B., and Myers, J., 1979. Behavioural and physiological adaptations of pea aphids (Homoptera: Aphididae) to high ground temperatures and predator disturbance. Can Entomol *111*, 515–519.

Roitberg, B.D., and Myers, J.H., 1978. Adaptation of alarm pheromone responses of the pea aphid *Acyrthosiphon pisum* (Harris). Can J Zool *56*, 103–108.

Roitberg, B., Myers, J., and Frazer, B.D., 1979. The influence of predators on the movement of apterous pea aphids between plants. J Anim Ecol *48*, 111–122.

Ruth, W.E., McNew, R.W., Caves, D.W., and Eikenbary, R.D., 1975. Greenbugs (Homoptera: Aphididae) forced from host plants by *Lysiphles testaceipes* (Hymenoptera: Braconidae). Entomophaga *20*, 65–72.

Sabath, M.D., Boughton, W.C., and Easteal, S., 1981. Expansion of the range of the introduced toad *Bufo marinus* in Australia from 1935 to 1974. Copeia 676–680.

Schaefer, P.W., Dysart, R.J., and Specht, H.B., 1987. North American distribution of *Coccinella septempunctata* (Coleoptera: Coccinellidae) and its mass appearance in coastal Delaware. Environ Entomol *16*, 368–373.

Sengonca, C., and Frings, B., 1985. Interference and competitive behavior of the aphid predators, *Chrysoperla carnea* and *Coccinella septempunctata* in the laboratory. Entomophaga *30*, 245–251.

Shanmuganathan, T., Pallister, J., Doody, S., McCallum, H., Robinson, T., Sheppard, A., Hardy, C., Halliday, D., Venables, D., Voysey, R., et al., 2010. Biological control of the cane toad in Australia: a review. Anim Conserv *13*, 16–23.

Sharov, A., n.d. Quantitative Population Ecology. Predator-prey model with functional and numerical responses. Accessed June 2013. http://home.comcast.net/~sharov/PopEcol/lec10/fullmod.html

Snyder, G.B., Finke, D.L., and Snyder, W.E., 2008. Predator biodiversity strengthens aphid suppression across single-and multiple-species prey communities. Biol Control *44*, 52–60.

Snyder, W.E., Clevenger, G.M., and Eigenbrode, S.D., 2004. Intraguild predation and successful invasion by introduced ladybird beetles. Oecol *140*, 559–565.

Stiling, P., and Simberloff, D., 2000. The frequency and strength of nontarget effects of invertebrate biological control agents of plant pests and weeds. In Nontarget Effects of Biological Control, P. Follet, and J.J. Duan, eds. (Kluwer Academic Publishers), pp. 32–43.

Valéry, L., Fritz, H., Lefeuvre, J.C., and Simberloff, D., 2008. In search of a real definition of the biological invasion phenomenon itself. Biol Invasions *10*, 1345–1351.

Vitousek, P., D'Antonio, C., Loope, L., and Westbrooks, R., 2006. Biological Invasions as Global Environmental Change. Amer Sci *84*, 468–478.

Vitousek, P.M., Mooney, H.A., Lubchenco, J., and Melillo, J.M., 1997. Human domination of Earth's ecosystems. Science *277*, 494–499.

Wheeler Jr, A., and Hoebeke, E., 1995. *Coccinella novemnotata* in northeastern North America: historical occurrence and current status (Coleoptera: Coccinellidae). Proc Entomol Soc Washington 103–111.

Wyatt, I.J., and White, P.F., 1977. Simple estimation of intrinsic increase rates for aphids and tetranychid mites. J Appl Ecol *14*, 757–766.

CHAPTER 2

The foraging behavior of two coccinellids, *Coccinella novemnotata* and *Coccinella septempunctata*, on the pea aphid (*Acyrthosiphon pisum*)

1.0 Introduction

1.1 Background

Classical biological control is a process that is used to reduce invasive pest populations by importing natural enemies of the pest species. If implemented correctly, biological control can offer sustained pest management at a low cost (Debach and Rosen, 1991; Barratt et al., 2000). However, if the control agents are not thoroughly researched prior to importation and release, they have a reduced potential to suppress pest populations and they may themselves become pests by attacking non-target organisms. It is estimated that 16% of all classical biological control projects in the United States have resulted in adverse non-target effects (Stiling & Simberloff, 2000).

Lady beetles are biological control agents that suppress populations of soft-bodied insects such as aphids in agricultural systems. One of the first successful biological control programs centered around the release of lady beetles, used the vedalia lady beetle, *Rodolia cardinalis* Mulsant, to control the cottony-cushion scale, *Icerya purchasi* Maskell, in citrus farms. The estimated cost of this program was about $5000 in 1888 and it has since saved the citrus industry millions of dollars annually (Debach and Rosen, 1991). Lady beetles are so successful as biological control agents, there have been many attempts to import them for pest suppression. During the twentieth century, at least 26 exotic lady beetle species have become established in the US, many of which were introduced intentionally (Gordon, 1985).

The seven-spotted lady beetle, *Coccinella septempunctata* L. was one of several coccinellids that was imported from Europe into the United States for the biological control of aphids during the twentieth century. There are numerous documented attempts made by the U.S. Department of agriculture to import this species, including a classical biological control program meant to control the Russian wheat aphid (*Diuraphis noxia* Mordvilko) (Schaefer et al., 1987; Obrycki et al., 2000). Populations of *C. septempunctata* in the US were first discovered 1973, after which they rapidly spread across the United States (Schaefer et al., 1987). The establishment of *C. septempunctata* has been purported to have had a profound impact on the relative abundances of native coccinellids, many of which have become significantly less common since its establishment (Elliott et al., 1996; Obrycki et al., 2000). For instance, the nine-spotted lady beetle, *Coccinella novemnotata* Herbst, which shares the same ecological niche and can often be found on plants also inhabited by *C. septempunctata* (Harmon et al., 2007; Losey et al., 2012), has seen a sharp decline in abundance. This is remarkable because prior to the establishment of *C. septempunctata, C. novemnotata* was one of the most commonly collected lady beetles in the United States (Gordon, 1985).

The replacement of *C. novemnotata* by *C. septempunctata* may have significant implications for aphid pest management in agricultural systems. Hoki et al. (2014) reported that *C. septempunctata* consumed more pea aphids, (*Acyrthosyphon pisum* Harris), at every density tested compared to *C. novemnotata. C. septempunctata* was so successful at consuming *A. pisum* that in computer simulations it often drove aphid populations to extinction, which indicated that it may have a less stable relationship with its prey than *C. novemnotata.* These results indicate that *C. septempunctata* has a larger consumptive effect on aphids than did *C. novemnotata,*

however that study considered prey exploitation once prey had been encountered and it did not address differences in their prey-finding capacity.

When looking at the overall impact of coccinellids on aphids, one important consideration is their ability to forage for aphids. If *C. septempunctata* is more efficient at finding prey and has a higher consumptive impact on aphids than *C. novemnotata*, this could indicate that it is much more effective as a biocontrol agent and as a competitor. This may also provide some insight into why *C. novemnotata* populations have dropped since *C. septempunctata* became established. Conversely, if *C. septempunctata* is less efficient at finding its prey than *C. novemnotata,* then it is possible that predation rate alone overestimates the differential in impact on aphid populations because the high consumptive impact is counteracted by poor foraging ability.

One factor that often has a substantial impact on foraging efficiency is the use of olfactory cues. There is ample evidence that coccinellids use olfactory cues to assist them in finding their prey while foraging (Obata, 1986; Obata, 1997; Abassi et al., 2000; Schaller and Nentwig, 2000; Ide et al., 2007). They are drawn to a variety of stimuli including herbivore-induced plant volatiles (Ninkovic et al., 2001; Pettersson et al., 2008), high aphid density (Hodek & Honek, 1996), aphid honeydew (Ide et al., 2007), and the aphid alarm pheromone, (E)-ß-Farnesene (Al Abassi et al., 2000; Verheggen et al., 2007). These stimuli may be important in both finding prey and determining lady beetle retention time (how long a coccinellid will stay at a specified site).

1.2 Objectives

We initiated this study to compare the foraging behavior of *C. novemnotata* and *C. septempunctata* on an important agricultural pest and primary prey species, *A. pisum*. Our objectives were 1) to quantify preference of location and retention time for single adult individuals of both species on plants with and without aphids, 2). to quantify preference of location and retention time for heterospecific and conspecific pairs of adult beetles, and, 3) to compare the role, if any, that olfactory cues play in medium range foraging across both species. These experiments give insight into how two congeneric coccinellids locate their prey, respond to aphid density, and interact with each other once that prey species is found.

2.0 Methods

2.1.0 Rearing Methods

C. septempunctata and *C. novemnotata* were reared in our laboratory and were maintained at 23 ± 2°C with a 16:8h (L:D) light cycle. Adult beetles and larvae were placed individually into 44mL cups that contained a single 6 x 2 cm piece of folded paper towel, and they were provided an *ad libitum* diet of *A. pisum* that had been produced on fava bean (*Vicia faba* L.). Additionally, two to four granules of bee-collected pollen (0.016-0.032 g), purchased from a health food store, were added to each cup as a dietary supplement. A more detailed description of the beetle colony history and rearing process can be found in Hoki et al. (2014). Because of the coccinellids' propensity to feed on the leaves (Ugine et al., unpublished), we provided each beetle with fava bean leaves the day before each experiment to reduce leaf feeding during data collection.

2.2.0 The effect of aphid density on lady beetle retention time

To determine the effect of lady beetle species and aphid density on the retention time of adult lady beetles on fava beans, we quantified the behavior of individual adult beetles on individual plants in small cages (35.5 x 35.5 x 70cm). Cages were placed into two adjacent greenhouse rooms which were kept at 23.2°C ± 0.12 with RH at 22.5% ± 0.5. The conditions of the rooms were recorded every 15min using a HOBO data logger (Onset, Bourne, MA). One 14d-old *V. faba* plant (approximately 15cm tall to the last node) contained within a 10cm diameter plastic pot was placed into each cage. Aphid density was set by infesting plants with 0, 50, or 250 mixed-aged pea aphids. We estimated the number of aphids for the 250 aphids per plant treatment by determining the weight of eight replicate groups of 50 aphids (mean weight = 0.465g) and then we weighted out five times that amount. The base of each plant was surrounded with a 44mL cup that had the bottom removed and that had been coated with Insect-a-Slip (BioQuip, Rancho Dominguez, CA). The radial cut in the cup allowed the barrier to be placed around the plant and later removed, with ease. This technique served to corral the aphids, which were added directly to the soil surface, near the plant stem and prevent their escape from the system. Aphids were added to the plants one day before the start of the experiment to allow them to distribute themselves and settle.

After 24h the cup-barriers were removed from around each plant and a single adult *C. novemnotata* or *C. septempunctata* (16.49 ± 0.78 and 17.32± 0.81 days old, respectively), which had been held for the 24h prior to the experiment with just fava bean leaves, was introduced to the base of the stem of each plant. We recorded the time that each beetle was introduced to each cage. The greenhouses were relatively small and it took no more than 1min to locate the position of all of the beetles within a greenhouse. The position of each beetle was monitored until it left

the plant or for 120min, and the time that each beetle left a plant was recorded. The experiment was conducted over a span of 7 weeks and equal numbers males and female beetles (for *C. septempunctata,* N = 80; for *C. novemnotata,* N = 82) were tested for each species. . We considered time spent on the cage, soil, or pot, as "leaving the plant."

2.3.0 The effect of heterospecific and conspecific coccinellid pairings on beetle location

To determine if other lady beetles within a system would influence a coccinellid's location and how that might affect foraging behavior we grouped conspecific and heterospecific pairs of *C. novemnotata* and *C. septempunctata* and recorded the location of each beetle in the presence of aphid-infested and aphid-free plants. By doing this, we hoped to determine how the beetles interacted with members of their own species as well as a different coccinellid species and how those interactions affected beetle location and foraging behavior.

Prior to the experiment, one-week old *V. faba* seedlings were transplanted into 500mL funnels filled with Lambert LM-series professional growing media (Quebec City, Canada). A 5cm wide coat of Insect-a-Slip (BioQuip, Rancho Dominguez, CA) was applied around the upper edge of each funnel to prevent the escape of aphids and coccinellid that dropped or walked to the soil surface. The funnels were placed into 10 cm diam. pots for added stability and moved into a green house kept at 23.9°C ± 0.17 with RH at 22.7% ± 0.5. When plants reached a height of 15cm (ground level to last node) they were moved into 14 x 14 x 24in rearing cages (BioQuip, Rancho Dominguez, CA) with two plants in each cage. Bamboo stakes (0.5cm diameter) were used to keep the plants upright during the trial. After the plants were placed in the cage, 50 third or fourth-instar aphids were added to one of the plants and the barrier (see section 2.2.1) was placed around its base. The aphids were allowed 24 hours to settle. The following day the barrier

was removed and the dead aphids that had not climbed up the plant were counted. A 12 x 4cm mesh bridge was placed between the two funnels, connecting them, while touching the soil in each funnel. This allowed the coccinellids easier access to the plant. Two adult virgin beetles (mean ± SE day old adults: 16.02 ± 0.80 days) were then placed onto the mesh bridge and allowed to move into the plants. The treatments were: two *C. septempunctata,* two *C. novemnotata,* or one *C. septempunctata* and one *C. novemnotata* per cage. To prevent the beetles from mating during the trials, each treatment consisted of two beetles of the same sex. Both sexes were tested to see if there were innate behavioral differences. We tested an equal number of male and female replicates with a total of 23 replicates per treatment.

The first plant each coccinellid came in contact with was recorded and the position of each beetle was recorded every 5 minutes for 2 hours. It was either marked as: "on the aphid-infested plant," "on the aphid-free plant," "on the cage," or "off the plant." For beetles not on either plant, the closest funnel was noted. Prior to this experiment, the coccinellids were weighed, starved for 24h, and marked with acrylic paint. *V. faba* leaves were provided 24h before the experiment to prevent plant feeding during the experiment.

2.4.0 Olfactory test

To compare the ability of each coccinellid species to locate prey using olfactory cues we conducted a Y-tube experiment. The Y-tube used in this experiment was made of glass and was 4cm in diameter, 27cm long at the neck and 14cm long at the arms. Each arm of the Y-tube was connected to a 3.76L mason jar where an odor source was placed. The odor source provided was either a plant infested with aphids, a plant without aphids, or an empty jar filled with 50mL of water. The water was added to the empty jar to match the humidity caused by the respiration of

plants from the other odor sources. The plants were planted in 10cm pots and were used when they reached 15cm in height (measured to the last node). The infested plants had been inoculated with aphids at least 52 hours prior to the experiment in order to elicit any herbivore induced plant volatiles (HIPV).

After placing the odor sources in the mason jars, we used two small aquarium pumps to move air through 1/4in ID tubes, to a flow meter, which was set to 0.8L per minute. From the flow meter, the tube was placed through the bottom of a 473mL container, which was fitted to the top of the mason jar forming an airtight seal (Figure 1). A second tube was place through the container and was attached to one of the arms of the Y-tube. The setup was the same on both sides of the Y-tube. To facilitate beetle movement, the Y-tube was directed upward at a 45°angle. Each beetle was denied aphids and starved for 24h prior to the experiment, however they were given access to fava bean leaves.

At the start of the experiment, a single beetle was placed at the base of the Y-tube. The beetle was given 5 minutes to move around the Y-tube where its location was recorded into three categories: right arm, left arm or neither. These partitions were divided at the intersection and were marked with a pen. Time spent near the base or in between the arms was marked as "neither". We also took note of which was the first plant the beetle moved towards and the where the beetle was at the end of the 5 minute period. If the beetle did not move towards either arm within the 5 minute period it was recorded as "no choice." To account for potential differences between the two Y-tube arms caused by external factors, we alternated the location of the odor source each time the trial was run. The conditions of the room were recorded every 15min using a HOBO data logger (Onset, Bourne, MA) for 5 weeks. The duration of the experiment, the room was kept at 22.5°C ± 0.23 with RH at 20.9% ± 0.5

We compared two pairs of treatments in the Y-tube set-up. The first test compared the beetle's olfactory response to a *V. faba* plant infested with aphids and a non-infested plant, we tested 56 beetles of both *C. septempunctata* and *C. novemnotata* with equal numbers of males and females. The second test measured the beetle's response to a plant with infested with aphids and an empty jar with 50mL water. Here we tested 52 beetles of both *C. septempunctata* (30 females, 22 males) and *C. novemnotata* (26 males and 26 females). For each combination we recorded what was the first choice made by the coccinellid, as well as how long they spent in arm of the Y-tube.

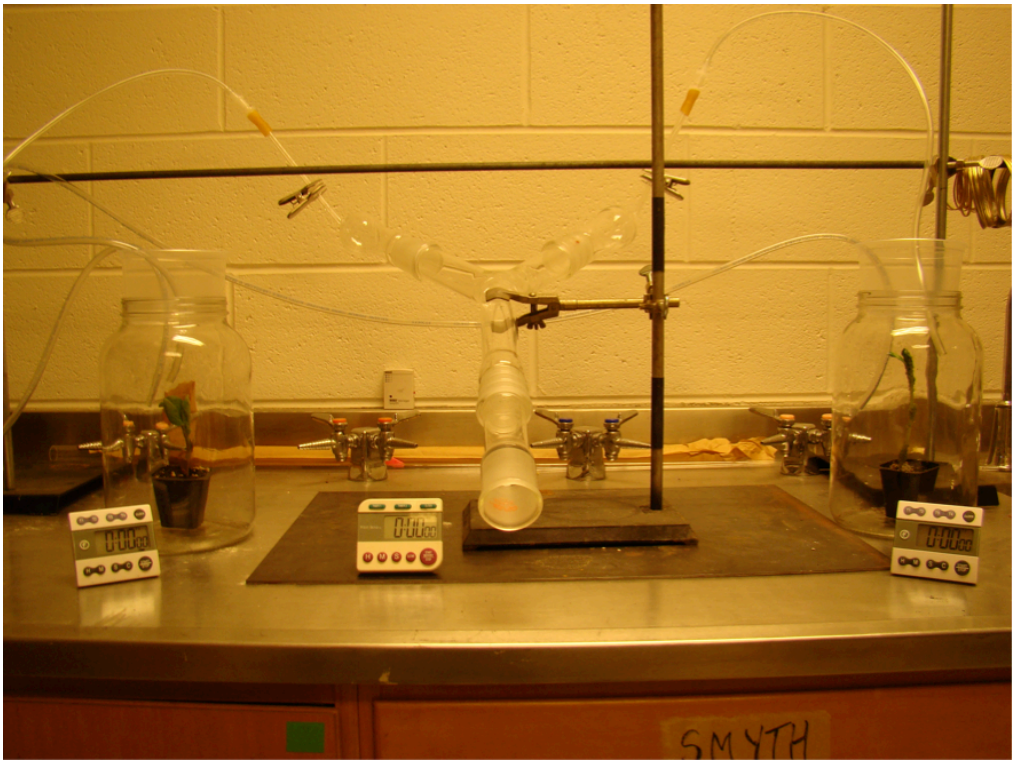

Figure 4: The Y-tube set up used in the experiment

2.5.0 Statistical methods

All statistical analyses were conducted using the statistical package JMP Pro 10 (SAS Institute, 2013). Retention times (number of minutes spent on the plant before leaving) of lady

beetles maintained singly within a cage were analyzed using proportional hazards regression. Fixed effects included lady beetle species, aphid density, sex, experimental date (block), and the interaction of lady beetle species and aphid density. Beetles that did not leave their plant during the 2h test period were right-censored for the analysis.

To test the effect of conspecific and heterospecific coccinellid pairings on beetle location we used a generalized linear model in JMP Pro 10 (SAS Institute, 2013) to measure the proportion of time spent on aphid infested or non-infested plants in relation to coccinellid pairing, sex, and species. We compared time spent by the coccinellids, both alone and together, on the aphid-infested plant and on either of the plants. The effect of the coccinellid pair combination, sex, and species were analyzed separately at each location. To correct for multiple comparisions, we used a post-priory Bonferroni correction (Sokal and Rohlf, 1995).

We ran two nominal logistic regressions to determine if there were differences in how each coccinellid species interacted with various odors. Because we were not interested in all pairwise comparisons we opted to do select two-sample pairwise tests. First we ran a nominal logistic regression on the number of instances where the coccinellid species spent more than 50% of its time on the aphid-infested plant. The second regression compared the first choice of each species. For this analysis, the first choice could be one of three options: no choice, non-aphid option, or aphid-infested plant. The non-aphid option consisted of either the 50mL water treatment or the non-infested plant.

3.0 Results

3.1.0 The effect of aphid density on lady beetle retention time

There was a significant effect of sex for both *C. septempunctata* and *C. novemnotata* ($x^2$2df= 16.20, $P < 0.0001$) with males spending 30.1% more time on each plant across all aphid densities than females. Additionally, there was a significant species by aphid density interaction ($x^2$2df= 6.279, $P = 0.043$) where *C. septempunctata* was more likely to leave a plant when no aphids were present then *C. novemnotata.* There was no significant effect of coccinellid species or aphid density on beetle retention time (x^2df= 1.781, $P = 0.182$; x22df= 4.768, $P = 0.092$, respectively).

3.2.0 The effect of coccinellid pair combinations on beetle location and retention time

The combination of species within a pair had a significant effect on how long each species spent on either plant (x^2df= 25.59, $P < 0.0001$) and on the aphid-infested plant ($x^2$2df= 40.20, $P < 0.0001$). Pairs from both species spent larger proportion of time on the aphid infested plant than at any other recorded location in the cage (Table 1). *C. novemnotata* pairings spent 45.23 ± 6.7% of their time on the aphid infested plants, significantly longer than the *C. septempunctata* pairings at 31.94 ± 5.2% (x^2df= 38.135 $P < 0.0001$). In contrast, *C. septempunctata* spent significantly more time on the non-infested plant than *C. novemnotata* (x^2df= 9.200, $P < 0.0001$) with values of 24.64 ± 5.6% and 18.50 ± 4.1% respectively.

The two species behaved differently in regard to how they interacted with other beetles. Beetles in the conspecific *C. novemnotata* pairings spent significantly more time together on the same plant than the conspecific *C. septempunctata* pairings at 32.32 ± 7.5% compared to 15.01 ± 4.6% (x^2df= 57.788, $P < 0.0001$). Additionally, the conspecific *C. novemnotata* beetles spent

significantly more time together on the same plant than beetles in the heterospecific trials, which spent 14.64 ± 3.9% of their time together (x^2df= 63.556, P <0.0001). Conversely, in the conspecific trails *C. septempunctata* spent more of their time together but not significantly more than in the heterospecific trials (x^2df= 0.854, P = 0.355).

Sex was an important factor when considering coccinellid location. Between the two sexes, there was no significant difference in the amount of time spent on the aphid-infested plant for either *C. septempunctata* (x^2df= 2.335, P = 0.127) or *C. novemnotata* (x^2df= 0.096, P = 0.757). However, on the non-infested plant, the two sexes behaved differently with females spending less time then males at that location. This pattern held true for both *C. septempuncatata* (x^2df= 4.952, P = 0.0261) and *C. novemnotata* (x^2df= 15.659, P < 0.0001).

Table 1: The proportion of time spent at each location by conspecific *C. novemnotata* pairs (C9-C9), *C. septempunctata* pairs (C7-C7), and heterospecific pairs (C7-C9)

Test	C9-C9	C7-C7	C7-C9
Aphid Plant	0.452 ± 0.07 A	0.319 ± 0.05 B	0.378 ± 0.42 B
Non-infested Plant	0.185 ± 0.04 A	0.246 ± 0.06 B	0.209 ± 0.04 AB
Either Plant	0.637 ± 0.06 A	0.566 ± 0.07 B	0.588 ± .043 B
Together (Aphid)	0.318 ± 0.07 A	0.150 ± 0.05 B	0.143 ± 0.04 B
Together (Non-infested)	0.110 ± 0.04 A	0.03 ± 0.01 B	0.034 ± 0.01 B
Together (Either)	0.208 ± 0.07 A	0.120 ± 0.05 B	0.109 ± 0.03 B

3.3.0 The olfactory test

The first experiment we conducted tested coccinellid preference for either water or a plant infested with aphids measuring the proportion of time spent over 50% in either arm of the

Y-tube and which side the coccinellid selected to go to first. We found no significant differences in any of the pairwise comparisons regarding species for either the proportion of time analysis ($P > 0.238$) or the first choice test ($P > 0.275$). Sex was not significantly different either with all values of $P > 0.105$ for the proportion time analysis, and $P > 0.407$ for the first choice tests. There was no significant effect of the sex by species interaction with values of $P > 0.288$ and $P > 0.081$ for the proportion of time analysis and the first choice analysis, respectively

The second experiment tested coccinellid preference for either non-infested or aphid-infested fava bean plants. We found no significant effect of species in any of the tests when we conducted pairwise comparisons of lady beetle preference for either of these stimuli. This was true for all of the first choice analyses ($P > 0.392$) and the proportion of time tests ($P > 0.203$). Sex did not impact preference either with values of $P > 0.602$ for the first choice test $P > 0.751$ for the proportion of time analysis. There was no interaction between sex and species after we corrected the values using the sequential Bonferroni procedure, with values of $P > 0.075$ and $P > 0.964$, for proportion time and first choice analysis, respectively.

4.0 Discussion

Our results show that *C. novemnotata* and *C. septempunctata* have different foraging behaviors in regard to their response to aphid density, and how the beetles interact during conspecific and heterospecific interactions. The differences in the foraging behavior help us to begin to understand how these two species may interact in the field, and how their interactions might impact aphid population dynamics. Our results represent an important expansion of earlier findings that *C. septempunctata* has a higher attack rate and handling time than *C. novemnotata* (Hoki et al. 2014).

In this study we found that conspecific and heterospecific interactions had a greater impact on the retention time of *C. novemnotata* than *C. septempunctata.* Conspecific pairs of *C. novemnotata* spent a significantly larger amount of time on aphid-infested plants than the conspecific *C. septempunctata* and the heterospecific pairings. Interestingly, the time spent by *C. septempunctata* on the aphid-infested plant was not significantly different than the heterospecific pairing. When looking at individual beetles in each trial, we found that *C. novemnotata* spent significantly more time on the aphid-infested plants when paired with a conspecific beetle then when paired with *C. septempunctata.* This could indicate that *C. novemnotata* is deterred by the presence of *C. septempunctata*. This could be for a variety of reasons. For instance, *C. septempunctata* is larger (Losey et al., 2012) and more voracious than *C. novemnotata* (Hoki et al., 2014) and frequently engages in intraguild predation and cannibalization events (Snyder et al., 2004; Kajita et al., 2006; Turnipseed et al., 2014 in press). It is possible that *C. novemnotata* exhibited avoidance behavior to reduce the possibility being preyed upon by *C. septempunctata.* Additionally, if *C. septempunctata* beetles were avoiding other conspecifics to reduce cannibalism, this would explain why conspecific pairs of *C. septempunctata* spent less time together than the less voracious *C. novemnotata.* Another possibility is that the two species were trying to avoid competition. Since *C. novemnotata* consumes less aphids and may have a more stable relationship with its aphid prey (Hoki et al., 2014) than *C. septempunctata*, they may be more willing to share the same space. In contrast, *C. septempunctata,* which consumes many more aphids than *C. novemnotata* (Hoki et al., 2014), could risk running out of prey if competition is too high. This hypothesis would explain why pairs of *C. novemnotata* spent more time together on the aphid-infested plant than *C. septempunctata.* Because of high competition and intraguild predation events, many coccinellids actively avoid each other (Hemptinne and

Dixon, 2000; Ninkovic et al., 2013). It is not surprising that *C. septempunctata* exhibits this behavior.

While it is important to see how conspecific and heterospecific pairs interact, we also wanted to investigate how individual beetles responded to aphid density and how that would impact their retention time. We found that the two species responded differently to aphid density, where *C. septempunctata* left the plants more quickly when no aphids were present than *C. novemnotata* (Figure 2) One possible explanation for this is that *C. septempunctata* generally consumes more aphids than *C. novemnotata* (Hoki et al., 2014) and since it is generally larger (Losey et al. 2012) it may require more food for development. If that were the case, it would make sense that *C. septempunctata* would quickly leave a plant where no prey was present.

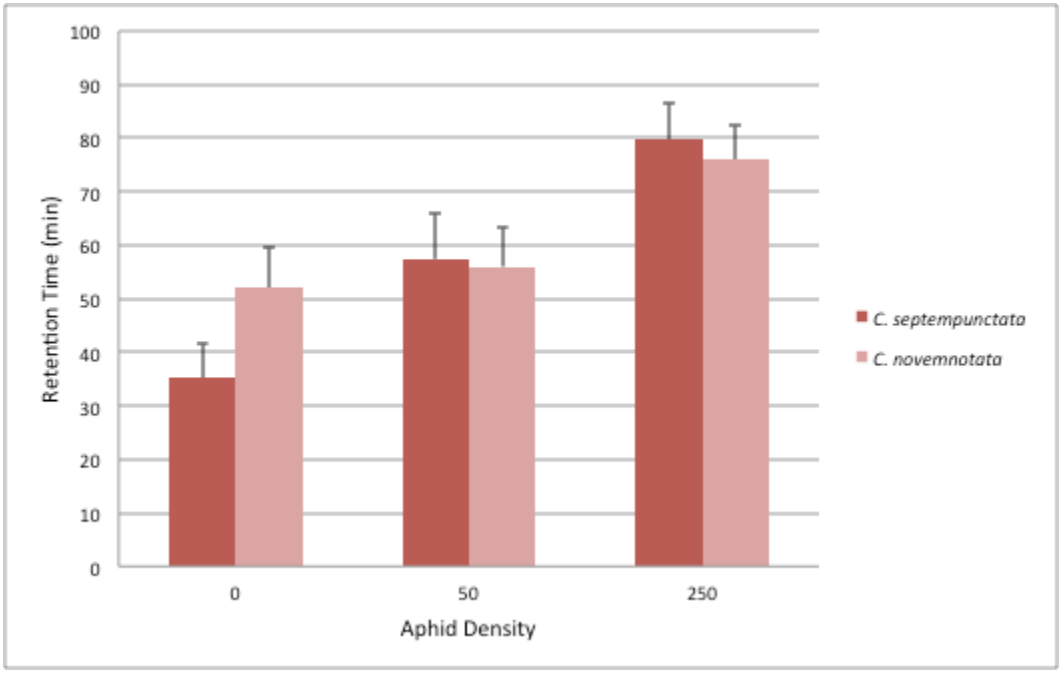

Figure 5: *C. septempunctata* and *C. novemnotata* retention time in response to aphid density

Combining examination of foraging decisions for single and multiple beetles allows us to assess the impact of beetle interactions on foraging. One emergent property is that the two coccinellid species behave very differently when in pairs rather than alone. For instance, one

experiment found that *C. septempunctata* leaves plants more quickly than *C. novemnotata* at lower aphid densities, yet the other experiment shows that in pairs, *C. septempunctata* pairs spends more time on non-infested (thus lower density) plants implying that they are slower to leave than *C. novemnotata.* This could be the result of the conspecific interactions between each species where *C. septempunctata* actively avoids spending time near other coccinellids. Since *C. novemnotata* prefers to spend time on the aphid-infested plant and has less of an aversion to spending time conspecific beetles, it makes sense that conspecific pairs of *C. novemnotata* would reside on the aphid-infested plants for a longer duration of time.

We found that pairs of female beetles of both coccinellid species spent less time than the males on the non-infested plant, but similar time on the aphid-infested plant. This female behavior could be a way of making sure the larvae have the enough resources once the eggs hatch. Interestingly, when alone, female beetles of both species spent less time on plants of all aphid densities than the males. The fact that the difference between sexes disappears on the aphid-infested plants when the beetles are paired together could indicate that both sexes deter each other through conspecific interactions, but the deterrence is stronger among males. Alternatively, the importance of aphid presence for females may be important enough to overcome their aversion to being at the same location as other conspecifics, whereas for males it is preferable to leave.

We found *C. septempunctata* females still spent less time than the *C. novemnotata* females on the aphid infested plant. If potential food for the larvae is so important, we would expect that *C. septempunctata* females would spend as much time as possible on the aphid-infested plants in order to deposit eggs. However, it is possible that intraguild predation, egg predation, and cannibalism are such great risks to larval development that they outweigh the

benefits of prey abundance. If this were the case, *C. septempunctata* females might avoid each other even in the presence of potential larval prey. Multiple studies have found that if coccinellids run through a resource they are more likely to cannibalize and engage in intraguild predation (Agarwala & Dixon, 1992; Hironori and Katsuhiro, 1997; Lucas et al., 1998). Turnipseed et al. (2014 in press) has demonstrated this in *C. novemnotata* and *C. septempunctata*. Since *C. septempunctata* exhibits higher feeding rates than *C. novemnotata* (Hoki 2014) it may be more likely to run out of food and engage in intraguild predation. Additionally, cannibalism increases with larval density (Mills, 1982). Eggs are also at risk since many coccinellids also engage in egg predation (Snyder et al., 2004; Kajita et al., 2010; Smith and Gardiner, 2013). The potential for cannibalism, competition, egg predation, and the interaction between these factors are strong motivators for female coccinellids to avoid each other, especially if there is a danger of running out of prey.

When assessing the efficacy of a biological control agent, it is important to take into consideration conspecific interactions. A study by Hoki et al. (2014) suggests that *C. septempunctata* may be a more effective biological control agent then *C. novemnotata* based on predation rates. However, that study is based on prey exploitation by single beetles and did not take into account the relative ability of the two species to locate groups of prey to exploit and it does not account for the conspecific interactions between beetles. Based on the results of this study, we conclude that although *C. septempunctata* is more effective on an individual basis, *C. novemnotata* may be just as effective as a control agent due to their toleration of other conspecific beetles. To demonstrate this idea we took the maximum attack rate values ($1/hT$) from Hoki et al. (2014) and created a ratio comparing *C. septempunctata* to *C. novemnotata*, which was 1.43. We then took a ratio based on the number of 5-minute intervals that each

species pair spent on the aphid-infested plant, comparing *C. novemnotata* to *C. septempunctata.* The ratio we found was 1.41. This provides evidence that while *C. septempunctata* is has an attack rate that is 43% higher, pairs of *C. novemnotata* spend 41% more time on aphid-infested plants allowing them to feed longer, increasing their efficacy as biological control agents. This could mean that the respective efficacy of *C. novemnotata* and *C. septempunctata* as biological control agents may be more similar than previously thought.

While we found that *C. novemnotata* and *C. septempunctata* both interact with each other and respond differently to aphid density, we were also interested in seeing if there was a difference in their usage of olfactory cues while foraging. Several studies have shown that coccinellids can use various olfactory cues such as herbivore-induced plant volatiles (Ninkovic et al., 2001; Pettersson et al., 2008), aphid honeydew (Ide et al., 2007), and the aphid alarm pheromone, (E)-ß-Farnesene (Nakamuta 1991; Al Abassi et al, 2000; Heptane, 2000; Francis et al., 2004). Although we cannot completely rule out the possibility, our results provide no support for the use of the olfactory cues provided by a single fava bean plant infested with *A. pisum* by these two coccinellid species. It would appear that the ability to discriminate between infested and non-infested plants arises from the utilization of non-olfactory cues.

The replacement of *C. novemnotata* and *C. septempunctata* could mean various things in regard to biological control in agricultural systems. Multiple studies have found that the introduced biological control agent, *C. septempunctata* consumes more aphids than many of its native counterparts (Hoki et al., 2014) and therefore may be more effective at controlling aphid pest species. This study has found that while this data may be correct on an individual basis, it is also important to consider the conspecific interactions of that species. We found that pairs of *C. septempunctata* spent less time around aphid populations than pairs of *C. novemnotata* because

of their propensity to avoid each other. If this pattern held true in the field, groups of *C. novemnotata* may be just as effective as *C. septempunctata* as natural enemies of aphid populations. Additionally we found that aphid density and coccinellid sex are important factors in regard to beetle location. Contrary to some studies (Obata, 1986; Obata, 1997; Abassi et al., 2000; Schaller and Nentwig, 2000; Ide et al., 2007) we found no evidence for the use of olfactory cues from a single fava bean plant infested with aphids by either C7 or C9. These experiments show some of the similarities and differences in the foraging behavior of *C. novemnotata* and *C. septempunctata.* This study also stresses the importance of considering factors other than consumption when assessing the efficacy of a biological control agent.

REFERENCES

Agarwala, B.K., Dixon, A.F.G. (1993). Why do ladybirds lay eggs in clusters. Functional ecology *7* 541-548.

Al Abassi, S., Birkett, M.A., Pettersson, J., Pickett, J.A., Wadhams, L.J., Woodcock, C.M. (2000). Response of the seven-spot ladybird to an aphid alarm pheromone and an alarm pheromone inhibitor is mediated by paired olfactory cells. Journal of Chemical Ecology *26*, 1765-1771.

Barrat, B., Ferguson, C., Goldson, S., Phillips, C., and Hannah, D. (2000). Predicting the risk from biological control agent introductions: A New Zealand Approach. In Nontarget Effects of Biological Control, (Kluwer Academic Publishers), pp. 60–75.

Debach, P., and Rosen, D. (1991). Biological control: definition and scope (Cambridge University Press).

Elliot, N., Kieckhefer, R., and Kauffman, W. (1996). Effects of an invading coccinellid on native coccinellids in an agricultural landscape. Oecol *105*, 537–544.

Gordon, R.D. (1985). The Coccinellidae (Coleoptera) of America North of Mexico. J New York Entomol S *93,* 1-912.

Harmon, J.P., Stephens, E., and Losey, J. (2007). The decline of native coccinellids (Coleoptera: Coccinellidae) in the United States and Canada. J Insect Conserv 85–94.

Hemptinne, J.-L., and Dixon, A. (2000). Defence, oviposition and sex: semiochemical parsimony in two species of ladybird beetles (Coleoptera, Coccinellida) ? A short review. Eur J Entomol 443–477.

Hironori, Y., Katsuhiro, S. (1997). Cannibalism and interspecific predation in two predatory ladybirds in relation to prey abundance in the field. Entomophaga *42* 153-163.

Hoki, E., Losey, J., and Ugine, T.A. (2014). Comparing the consumptive and non-consumptive effects of a native and introduced lady beetle on pea aphids (Acyrthosiphon pisum). Biol Control *70*, 78–84.

Ide, T., Suzuki, N., Katayama, N. (2007). The use of honeydew in foraging for aphids by larvae of the ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Ecol Entomol *32,* 455-460.

Kajita, Y., Takano, F., Yasuda, H., and Evans, E.W. (2006). Interactions between introduced and native predatory ladybirds (Coleoptera, Coccinellidae): factors influencing the success of species introductions. Ecol Entomol *31*, 58–67.

Kajita, Y., Obrycki, J.J., Sloggett, J.J., Haynes, K.F. (2010) Intraspecific alkaloid variation in ladybird eggs and its effects on con- and hetero-specific intraguild predators. Oecologia *163,* 313-322.

Losey, J., Perlman, J., Kopco, J., Ramsey, S., Hesler, L., Evans, E., Allee, L., and Smyth, R. (2012). Potential causes and consequences of decreased body size in field populations of Coccinella novemnotata. Biol Control *61*, 98–103.

Lucas, E., Coderre, D., Brodeur, J. (1998). Intraguild predation among aphid predators: Characterization and influence of extraguild prey density. Ecology *79* 1084-1092.

Mills, N.J. (1982). Voracity, cannibalism and coccinellid predation. Ann Appl Biol *101* 144-148.

Ninkovic, V., Al Abassi, S., and Pettersson, J. (2001). The Influence of Aphid-Induced Plant Volatiles on Ladybird Beetle Searching Behavior. Biol Control *21*, 191–195.

Obata, S. (1986). Mechanisms of prey finding in the aphidophagous ladybird beetle*, Harmonia axyridis* [Coleoptera: Coccinellidae]. Entomophaga *31*, 303–311.

Obata, S. (1997). The influence of aphids on the behavior of adults of the ladybird beetle, *Harmonia axyridis* (Col: Coccinellidae). Entomophaga *42*, 103-106.

Obrycki, J.J., Elliot, N.C., and Giles, K.L. (2000). Coccinellid introductions: potential for and evaluation of nontarget effects. In Nontarget Effects of Biological Control, P.A. Follet, and J.J. Duan, eds. (Kluwer Academic Publishers).

Pettersson, J., Ninkovic, V., Glinwood, R., Abassi, S.A., Birkett, M., Pickett, J., and Wadhams, L. (2008). Chemical stimuli supporting foraging behaviour of Coccinella septempunctata L. (Coleoptera: Coccinellidae): volatiles and allelobiosis. Appl Entomol Zool *43*, 315–321.

Schaller, M., Nentwig, W. (2000). Olfactory orientation of the seven-spot ladybird beetle, Coccinella septempunctata (Coleoptera : Coccinellidae): Attraction of adults to plants and conspecific females. Eur J Entomol *97*, 155-159.

Schaefer, P.W., Dysart, R.J., and Specht, H.B. (1987). North American distribution of Coccinella septempunctata (Coleoptera: Coccinellidae) and its mass appearance in coastal Delaware. Environ Entomol *16*, 368–373.

Smith, C.A., Gardiner, M.M. (2013). Biodiversity Loss following the Introduction of Exotic Competitors: Does Intraguild Predation Explain the Decline of Native Lady Beetles? PLoS One 8, 235-245.

Snyder, W.E., Clevenger, G.M., and Eigenbrode, S.D. (2004). Intraguild predation and successful invasion by introduced ladybird beetles. Oecol *140*, 559–565.

Sokal, R.R., and F.J. Rohlf. 1995. Biometry: The principles and practice of statistics in biological research. 3rd edition. W.H. Freeman, New York.

Stiling, P., and Simberloff, D. (2000). The frequency and strength of nontarget effects of invertebrate biological control agents of plant pests and weeds. In Nontarget Effects of Biological Control, P. Follet, and J.J. Duan, eds. (Kluwer Academic Publishers), pp. 32–43.

Turnipseed, R., Ugine T., and Losey, J.E. (2014). Effect of prey limitation on competitive interactions between a native lady beetle, *Coccinella novemnotata,* and invasive lady beetle, *Coccinella septempunctata* (coleoptera: coccinellidae). Environmental Entomology.

Verheggen, F.J., Fagel, Q., Heuskin, S., Lognay, G., Francis, F., Haubruge, E. (2007). J Chem Ecol *33,* 2148-2155.